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Remarks:

The sequence listing, which is published as annex to the application documents, was filed after the date of filing. The applicant has declared that it does not include matter which goes beyond the content of the application as filed.

(54) **Genetically modified plants and plant cells comprising heterologous heavy metal transport and complexation proteins**

(57) The present invention relates to genetically modified plants and plant cells, comprising nucleotide

sequences encoding heterologous heavy metal transport protein.

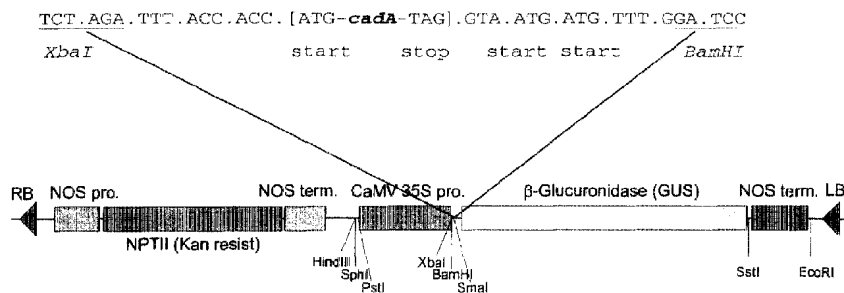


Fig. 1

Description

Field of the invention

[0001] The present invention is in the field of genetically modified plants and plants cells having improved heavy metal tolerance and accumulation due to increased plant growth and biomass production based upon the expression of exo-cytoplasmic heavy metal resistance system (efflux and complexation).

[0002] More particularly, the present invention is related to genetically modified plants and plant cells, comprising nucleotide sequences encoding heterologous heavy metal transport proteins and exocyttoplasmic metal binding proteins of various origins.

Background of the invention and state of the art

[0003] Heterologous nucleic acid sequences, coding for heavy metal resistance, were functionally expressed in plants, to improve their tolerance against these toxic elements. The heterologous heavy metal resistance genes, in casu represent either heavy metal efflux systems or functions involved in heavy metal sequestration.

[0004] Until present, only cytoplasmic functions that provide increased heavy metal resistance were expressed in plants :

1. Expression of heterologous metallothionein and phytochelatin in plants

[0005] Metallothioneins and phytochelatin, which are rich in cystein sulfhydryl residues that bind and sequester heavy metal ions in very stable complexes (Karin, 1985), are found in eukaryotic organisms, but recently also in *Syn-echococcus*. Various MT genes - mouse MTI, human MTIA (alpha domain), human MTII, Chinese hamster MTII, yeast CUP1, pea PsMTA - have been transferred to tobacco, cauliflower or *Arabidopsis thaliana* (Lefebvre et al., 1987; Maiti et al., 1988, 1989, 1991; Misra and Gedamu, 1989; Evans et al., 1992; Yeargan et al., 1992; Brandle et al., 1993; Pan et al., 1993; Elmayan and Tepfer, 1994; Hattori et al., 1994; Pan et al., 1994a, b; Hasegawa et al., 1997). As a result, varying degrees of enhanced Cd tolerance have been achieved, being maximally 20-fold compared with the control. Metal uptake levels were not dramatically changed; in some cases there were no differences, in others maximally 70% less or 60% more Cd was taken up by the shoots or leaves. Only one study has been reported on a transgenic plant generated with MT of plant origin. When pea (*Pisum sativum*) MT-like gene PsMTA was expressed in *Arabidopsis thaliana*, more Cu (several-fold in some plants) accumulated in transformed than in control plants (Evans et al., 1992).

2. Heterologous expression of heavy metal reduction

[0006] The only example known is the *mer* operon of *Tn21* of *Shigella flexneri*, whose expression in plants results in the reduction mercury (Hg^{2+}) in its metallic form (Hg^0). This metallic mercury is volatilized out of the cell (Rugh *et al.* 1996).

Aims of the invention

[0007] The present invention aims to provide a new way in obtaining plants and plant cells with improved heavy metal tolerance characteristics, and possibly heavy metal accumulation.

[0008] Another aim of the present invention is to provide such plants and plant cells which allow increased heavy metal resistance for revegetation and phytostabilisation of heavy metal contaminated sites.

[0009] A further aim of the present invention is to provide plants and plant cells, characterised by increased heavy metal accumulation combined with increased heavy metal tolerance which allow phytoextraction of heavy metals (inclusive rhizofiltration).

[0010] A last aim of the present invention is to provide a method which results in the possibility to improve important agriculture crop species with high biomass production in their heavy metal tolerance and accumulation.

Summary of the invention

[0011] The present invention is related to genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transport and/or sequestration proteins of various prokaryotic or eukaryotic origins.

[0012] Said transporters are preferably membrane proteins, which result in reduced toxicity due to the efflux of heavy metals from the cells, being preferably selected from the group consisting of P-type ATPases, 3 component efflux

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pumps, ABC transporters and CDF proteins (Cation Diffusion Facilitator proteins).

[0013] The family of the P-type ATPases is preferred, because of their advantage that for functional resistance only one protein is required.

[0014] Said proteins are found in both prokaryotic and eukaryotic organisms including plants.

[0015] Another advantage of said transporters is found as resistance mechanisms against many toxic trace elements of environmental concern, such as copper, cadmium, lead, zinc and silver.

[0016] Unexpectedly, it was not necessary to make structural changes in the coding sequence of said proteins, like it is necessary for the *merA* gene in order to obtain functional expression in plants (Rugh et al., 1996).

[0017] Preferably, the gene incorporated in the plants or plant cells is a gene encoding a bacterial P-type ATPase, preferably the cadmium ATPase, such as the *cadA* gene.

[0018] According to a second embodiment of the present invention, the system is based upon a prokaryotic heavy metal sequestration system, such as the *pcoA* family protein (more preferably the *pcoA* gene).

[0019] The various nucleotide sequences encoding heterologous heavy metal transport proteins can be deleted partially from non-specific nucleotide sequences which are not involved in efficient heavy metal transport or accumulation.

[0020] Said genetic sequences could be incorporated in a vector for the transfection of said plants or plant cells, such as the pBI121 vector, as described in the figure 1, said vector being advantageously an *E. coli/Agrobacterium*/plant shuttle vector, said vector comprising preferably a CaMV 35S promoter (a strong promoter constitutively expressed in plants).

[0021] Preferably, the system was introduced in the plants, such system allowing the transformation of plants with the *Agrobacterium tumefaciens* technology.

Short description of the drawings

[0022] Fig. 1 is a schematic representation of the cloning of *cadA* in pBI121.

[0023] Fig. 2 is a leaf disk-test with Nt WT SR1 (wild type), Nt PBI14 (pBI121) and Nt Cd 309 (pBI121-*cadA*) on 350 μ M Cd and control medium without Cd.

[0024] Fig. 3 represents the regeneration and growth of Nt WT SR1 (wild type), Nt PBI14 (pBI121) and Nt Cu122 (pBI121-*pcoA*) on 100 μ M Cu, the plant growth being shown from above (left) and top (right).

Detailed description of the invention

Heterologous expression of *cadA*

[0025] The heavy metal efflux system was CadA, a member of the P-type heavy metal efflux ATPase family of proteins found both in prokaryotic and eukaryotic organisms. P-type ATPases are all cation pumps, either for uptake, for efflux or for cation exchange. These enzymes have a conserved aspartate residue that is transiently phosphorylated from ATP during the transport cycle, hence the name 'P-type' ATPase (Silver et al., 1993).

[0026] The *cadA* gene from *Staphylococcus aureus* was amplified by PCR and cloned in the pBI121 vector.

[0027] During PCR, appropriate plant specific translation signals were added as well as XbaI and BamHI restriction sites, allowing cloning of the insert in the correct orientation.

[0028] The *cadA* fragment was cloned in the *Escherichia coli/Agrobacterium/plant* shuttle vector pBI121. In this vector, *cadA* expression is derived from the CaMV35S promoter, a strong promoter constitutively expressed in plants. The system was introduced in the plant *Nicotiana tabacum* cv. Petit Havana line SR1 via an *Agrobacterium tumefaciens* transformation (Horsch et al., 1985). The selection marker used was kanamycine.

[0029] Kanamycine resistant transformants were obtained after transformation. All the kanamycine resistant transformants tested showed an increased resistance to cadmium (tested by a leaf disk assay) compared to the wild type and transformant with the pBI121 vector without gene (fig. 1). This proves that the CadA P-type ATPase can be functionally expressed in plants, resulting in an increased resistance of the plant to the trace element (in casu cadmium).

[0030] It can be expected that for other members of the P-type ATPase family, which form a family of closely related proteins (both structural and functional) the same positive effect on resistance to specific trace elements will be found. Until present, P-type ATPases from both prokaryotic and eukaryotic have been identified that were found to interact with Zn, Cd, Pb, Cu and Ag (see table 1). It can not be excluded that P-type ATPases, encoding resistance to other trace elements including radioisotopes, will be identified.

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Table 1:

<i>different representatives of the family of P-type ATPases, from prokaryotic and eukaryotic origin, which encode resistance against trace elements such as Zn, Cd, Pb, Cu and Ag.</i>			
Gene	Sequence ID	Metals	Reference
CadA	P20021	Cd, Zn and Pb	Nucifora <i>et al.</i> 1989 Rensing <i>et al.</i> 1998
ZntA	P37617	Zn and Pb	Rensing <i>et al.</i> 1997 Rensing <i>et al.</i> 1998
CopF	Non available	Cu	van der Lelie and Borremans unpublished
PbrA	Not available	Pb	Borremans <i>et al.</i> , 2000
SilP	AF067954, nucleotide sequence <i>sil</i> operon	Ag	Gupta <i>et al.</i> , 1999
Menkes' disease	Q04656	Cu	Vulpe <i>et al.</i> 1993
Wilsos' disease	U08344	Cu	Pethrukin <i>et al.</i> 1993

Heterologous expression of *pcoA*

[0031] The other heavy metal resistance system is involved in exo-cytoplasmic heavy metal sequestration. The tested gene here was *pcoA* from *Escherichia coli* (Brown *et al.*, 1995), which was also cloned in pBI121 and introduced in *Nicotiana tabacum* through an *Agrobacterium tumefaciens* transformation in a way similar as described for *cadA*. Kanamycine resistant transformants were obtained after transformation. All the kanamycine resistant transformants tested showed an increased resistance to copper (tested by a leaf disk assay) compared to the wild type and transformant with the pBI121 vector without gene (Fig. 3).

[0032] The *pcoA* protein has many closely related members, found to be involved in resistance against Cu. In addition, other proteins of these copper resistance determinants have also been shown to be involved in Cu sequestration, such as PcoC/CopC and CopE. These proteins, although different in structure, are also active in the bacterial periplasm and possess similar heavy metal binding sites as *pcoA*. In addition, a CopE like protein, referred to as SilE, was identified in the *Salmonella sil* operon encoding for Ag-resistance. The potential genes whose heterologous expression can result in improved resistance, are summarised in table 2.

Genes	Sequence ID	Metals	References
<i>cop</i> operon (<i>copA</i> , C) e.g. of <i>Pseudomonas syringae</i>	M19930	Cu	Mellano and Cooksey (1988)
<i>pco</i> operon (<i>pcoA</i> , C) of e.g. <i>E. coli</i>	G619126	Cu	Brown <i>et al.</i> , 1995
<i>PcoE</i>	X83541	Cu	Brown <i>et al.</i> , 1995
<i>sil</i> operon of <i>Salmonella</i>	AF067954, nucleotide sequence <i>sil</i> operon	Ag	Gupta <i>et al.</i> , 1999

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[0033]

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Annex to the application documents - subsequently filed sequences listing

[0034]

5

SEQUENCE LISTING

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<110> Vlaamse Instelling voor Technologisch Onderzoek (V

<120> GENETICALLY MODIFIED PLANTS AND PLANT CELLS COMPRISING
HETEROLOGOUS HEAVY METAL TRANSPORT AND COMPLEXATION
PROTEINS

<130> P.VITO.25/EP

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<141> 2000-03-22

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<170> PatentIn Ver. 2.1

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<220>

<221> misc_feature

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<223> BamH I Restriction site

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sites and plant specific translation signals.

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 10 sites and plant specific translation signals.

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 Met Ser Glu Gln Lys Val Lys Leu Met Glu Glu Glu Met Asn Val Tyr
 1 5 10 15
 15 Arg Val Gln Gly Phe Thr Cys Ala Asn Cys Ala Gly Lys Phe Glu Lys
 20 20 25 30
 Asn Val Lys Lys Ile Pro Gly Val Gln Asp Ala Lys Val Asn Phe Gly
 35 40 45
 Ala Ser Lys Ile Asp Val Tyr Gly Asn Ala Ser Val Glu Glu Leu Glu
 25 50 55 60
 Lys Ala Gly Ala Phe Glu Asn Leu Lys Val Ser Pro Glu Lys Leu Ala
 65 70 75 80
 30 Asn Gln Thr Ile Gln Arg Val Lys Asp Asp Thr Lys Ala His Lys Glu
 85 90 95
 Glu Lys Thr Pro Phe Tyr Lys Lys His Ser Thr Leu Leu Phe Ala Thr
 35 100 105 110
 Leu Leu Ile Ala Phe Gly Tyr Leu Ser His Phe Val Asn Gly Glu Asp
 115 120 125
 40 Asn Leu Val Thr Ser Met Leu Phe Val Gly Ser Ile Val Ile Gly Gly
 130 135 140
 Tyr Ser Leu Phe Lys Val Gly Phe Gln Asn Leu Ile Arg Phe Asp Phe
 45 145 150 155 160
 Asp Met Lys Thr Leu Met Thr Val Ala Val Ile Gly Ala Thr Ile Ile
 165 170 175
 50 Gly Lys Trp Ala Glu Ala Ser Ile Val Val Ile Leu Phe Ala Ile Ser
 180 185 190
 Glu Ala Leu Glu Arg Phe Ser Met Asp Arg Ser Arg Gln Ser Ile Arg
 55 195 200 205

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	Ser	Leu	Met	Asp	Ile	Ala	Pro	Lys	Glu	Ala	Leu	Val	Arg	Arg	Asn	Gly	
	210						215					220					
5	Gln	Glu	Ile	Ile	Ile	His	Val	Asp	Asp	Ile	Ala	Val	Gly	Asp	Ile	Met	
	225					230					235					240	
10	Ile	Val	Lys	Pro	Gly	Glu	Lys	Ile	Ala	Met	Asp	Gly	Ile	Ile	Val	Asn	
					245					250					. 255		
	Gly	Leu	Ser	Ala	Val	Asn	Gln	Ala	Ala	Ile	Thr	Gly	Glu	Ser	Val	Pro	
15					260				265						270		
	Val	Ser	Lys	Ala	Val	Asp	Asp	Glu	Val	Phe	Ala	Gly	Thr	Leu	Asn	Glu	
			275					280					285				
20	Glu	Gly	Leu	Ile	Glu	Val	Lys	Ile	Thr	Lys	Tyr	Val	Glu	Asp	Thr	Thr	
	290						295					300					
	Ile	Thr	Lys	Ile	Ile	His	Leu	Val	Glu	Glu	Ala	Gln	Gly	Glu	Arg	Ala	
25	305					310					315					320	
	Pro	Ala	Gln	Ala	Phe	Val	Asp	Lys	Phe	Ala	Lys	Tyr	Tyr	Thr	Pro	Ile	
					325					330					335		
30	Ile	Met	Val	Ile	Ala	Ala	Leu	Val	Ala	Val	Val	Pro	Pro	Leu	Phe	Phe	
					340				345					350			
	Gly	Gly	Ser	Trp	Asp	Thr	Trp	Val	Tyr	Gln	Gly	Leu	Ala	Val	Leu	Val	
35			355					360				365					
	Val	Gly	Cys	Pro	Cys	Ala	Leu	Val	Ile	Ser	Thr	Pro	Ile	Ser	Ile	Val	
		370					375					380					
40	Ser	Ala	Ile	Gly	Asn	Ala	Ala	Lys	Lys	Gly	Val	Leu	Val	Lys	Gly	Gly	
	385					390					395					400	
	Val	Tyr	Leu	Glu	Lys	Leu	Gly	Ala	Ile	Lys	Thr	Val	Ala	Phe	Asp	Lys	
45					405					410					415		
	Thr	Gly	Thr	Leu	Thr	Lys	Gly	Val	Pro	Val	Val	Thr	Asp	Phe	Glu	Val	
					420				425					430			
50	Leu	Asn	Asp	Gln	Val	Glu	Glu	Lys	Glu	Leu	Phe	Ser	Ile	Ile	Thr	Ala	
			435					440					445				
	Leu	Glu	Tyr	Arg	Ser	Gln	His	Pro	Leu	Ala	Ser	Ala	Ile	Met	Lys	Lys	
55		450					455					460					

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	Ala	Glu	Gln	Asp	Asn	Ile	Pro	Tyr	Ser	Asn	Val	Gln	Val	Glu	Glu	Phe
	465						470					475				480
5																
	Thr	Ser	Ile	Thr	Gly	Arg	Gly	Ile	Lys	Gly	Ile	Val	Asn	Gly	Thr	Thr
					485					490					495	
	Tyr	Tyr	Ile	Gly	Ser	Pro	Lys	Leu	Phe	Lys	Glu	Leu	Asn	Val	Ser	Asp
10				500					505					510		
	Phe	Ser	Leu	Gly	Phe	Glu	Asn	Asn	Val	Lys	Ile	Leu	Gln	Asn	Gln	Gly
			515					520					525			
15																
	Lys	Thr	Ala	Met	Ile	Ile	Gly	Thr	Glu	Lys	Thr	Ile	Leu	Gly	Val	Ile
		530					535					540				
	Ala	Val	Ala	Asp	Glu	Val	Arg	Glu	Thr	Ser	Lys	Asn	Val	Ile	Gln	Lys
20	545					550					555					560
	Leu	His	Gln	Leu	Gly	Ile	Lys	Gln	Thr	Ile	Met	Leu	Thr	Gly	Asp	Asn
					565					570					575	
25																
	Gln	Gly	Thr	Ala	Asn	Ala	Ile	Gly	Thr	His	Val	Gly	Val	Ser	Asp	Ile
				580					585					590		
	Gln	Ser	Glu	Leu	Met	Pro	Gln	Asp	Lys	Leu	Asp	Tyr	Ile	Lys	Lys	Met
30			595					600					605			
	Gln	Ser	Glu	Tyr	Asp	Asn	Val	Ala	Met	Ile	Gly	Asp	Gly	Val	Asn	Asp
			610				615					620				
35																
	Ala	Pro	Ala	Leu	Ala	Ala	Ser	Thr	Val	Gly	Ile	Ala	Met	Gly	Gly	Ala
	625					630					635					640
	Gly	Thr	Asp	Thr	Ala	Ile	Glu	Thr	Ala	Asp	Ile	Ala	Leu	Met	Gly	Asp
					645					650					655	
40																
	Asp	Leu	Ser	Lys	Leu	Pro	Phe	Ala	Val	Arg	Leu	Ser	Arg	Lys	Thr	Leu
				660					665					670		
	Asn	Ile	Ile	Lys	Ala	Asn	Ile	Thr	Phe	Ala	Ile	Gly	Ile	Lys	Ile	Ile
45			675					680					685			
	Ala	Leu	Leu	Leu	Val	Ile	Pro	Gly	Trp	Leu	Thr	Leu				

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Claims

1. Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transporters or sequestration proteins.
2. Genetically modified plants or plant cells, the nucleotide sequence encoding the heterologous heavy metal transport proteins being genes encoding heavy metal transporters, such as transporters selected from the group consisting of P-type ATPase, 3 components efflux pumps or ABC transporters.
3. Genetically modified plants or plant cells according to the claim 2, **characterised in that** the nucleotide sequence encodes for cadmium ATPase.
4. Genetically modified plants or plant cells according to the claim 2 or 3, wherein the nucleotide sequence is *cad A* or a portion thereof allowing heavy metal transport.
5. Genetically modified plants or plant cells according to the claim 1, **characterised in that** the nucleotide sequence encoding the heavy metal sequestration protein belongs to the copA family.
6. Use of the genetically modified plants or plant cells according to any of the preceding claims for phytoremediation of contaminated sites, especially for the revegetation, phytostabilisation, phytoextraction of soils and/or water contaminated with trace elements.

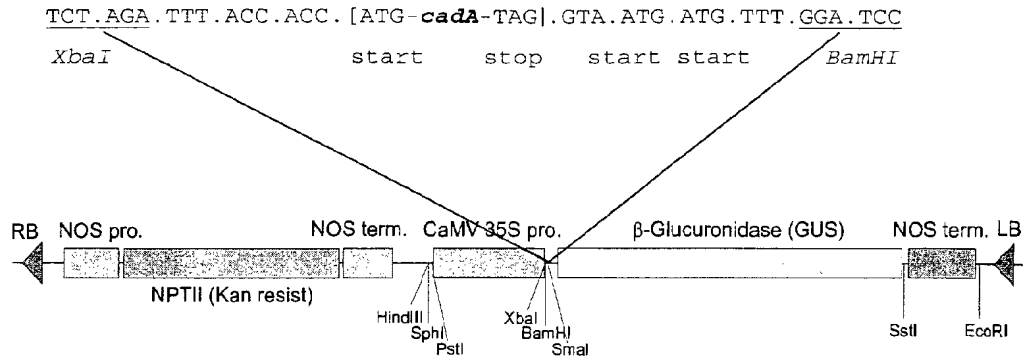


Fig. 1

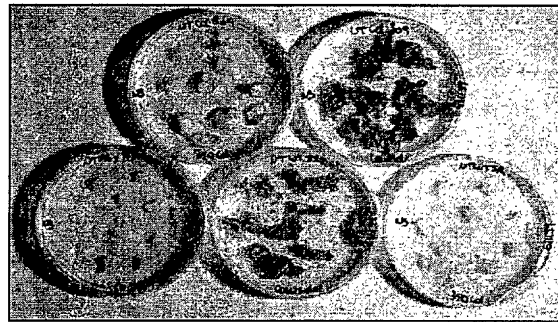


Fig. 2

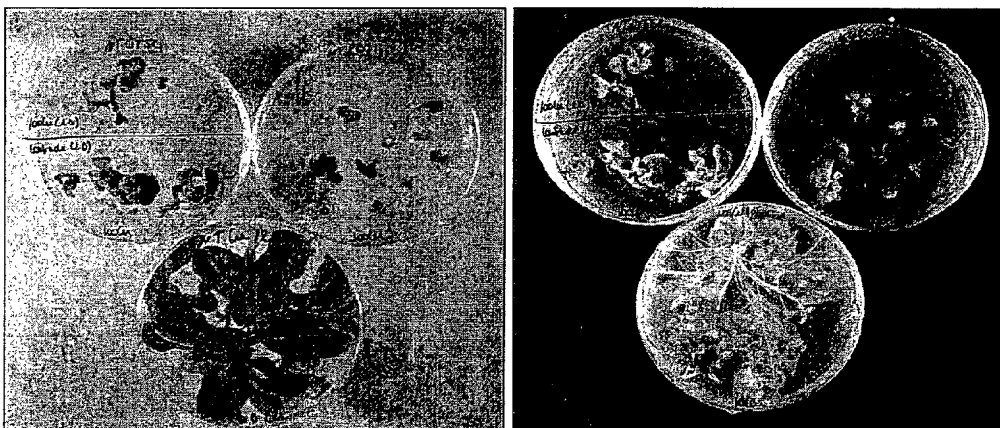


Fig. 3



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 87 0051

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	OW, D.W.: "Heavy metal tolerance genes: prospective tools for bioremediation" RESOURCES, CONSERVATION AND RECYCLING, vol. 18, no. 1, 1 November 1996 (1996-11-01), pages 135-149, XP004016621	1,2,6	C12N15/31 C12N15/82 C07K14/195 A01H5/00
A	* abstract * * page 137, line 1 - line 12 * * page 138, line 32 - page 140, line 7 * * page 147, line 16 - page 148, line 15 * ---	3,4	
X	WO 00 04760 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 3 February 2000 (2000-02-03)	1,2	
A	* abstract * * page 7, line 1 - line 5 * * page 8, line 18 - page 9, line 10 * * page 9, line 25 - line 32 * * page 11, line 1 - line 7 * * page 15, line 3 - line 12 * * page 41 - page 47; example 4 * * page 55 - page 58; claims 1,2,9,10,15,16,26 * ---	3,4,6	
X	WO 97 45000 A (TRUSTEES OF DARTMOUTH COLLEGE; REGENTS OF THE UNIVERSITY OF MINNESOTA) 4 December 1997 (1997-12-04)	1,6	C12N C07K A01H
A	* abstract * * page 2, line 4 - page 7, line 14 * * page 10, line 1 - page 13, line 14 * * page 36, line 34 - page 38, line 23 * * page 108 - page 110; claims 25,26,30,31,40,41,46-49 * --- -/-	2-4	
-The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 28 August 2000	Examiner Fuchs, U
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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Application Number

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1 and 6 partially and 2-4 completely



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 87 0051

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 98 04700 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 5 February 1998 (1998-02-05)	1,6	
A	* abstract * * page 1, line 8 - line 20 * * page 2, line 3 - line 7 * * page 2, line 30 - page 3, line 19 * * page 7, line 22 - line 24 * * page 8, line 28 - page 9, line 9 * * page 16, line 26 - page 18, line 12 * * page 26 - page 28; claims 1,5-7,10-12,14-19,23-26 *	2-4	
D,A	NUCIFORA, G. ET AL.: "Cadmium resistance from Staphylococcus aureus plasmid pI258 cad A gene results from a cadmium-efflux ATPase" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, no. 10, May 1989 (1989-05), pages 3544-3548, XP000938702 * the whole document *	1-4,6	
A	SILVER, S. & PHUNG, L.T.: "Bacterial Heavy Metal Resistance: New Surprises" ANNUAL REVIEW OF MICROBIOLOGY, vol. 50, 1996, pages 753-789, XP000925885 * the whole document *	1-4,6	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 28 August 2000	Examiner Fuchs, U
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 03.82 (P04C01)



European Patent
Office

**LACK OF UNITY OF INVENTION
SHEET B**

Application Number
EP 00 87 0051

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1 and 6 partially and 2-4 completely

Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transporters; said nucleotide sequences encoding heavy metal transporters selected from the group consisting of P-type ATPases, 3 components efflux pumps or ABC transporters; said nucleotide sequence encoding cadmium ATPase; said nucleotide sequence being cadA or a portion thereof allowing heavy metal transport and use of said plants or plant cells for phytoremediation of contaminated sites, revegetation, phytostabilisation, phytoextraction of soils / water contaminated with trace elements.

2. Claim : 1 and 6 partially and 5 completely

Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal sequestration proteins; said nucleotide sequences encoding heavy metal sequestration proteins belonging to the copA family and use of said plants or plant cells for phytoremediation of contaminated sites, revegetation, phytostabilisation, phytoextraction of soils / water contaminated with trace elements.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 00 87 0051

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-08-2000

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0004760	A	03-02-2000	NONE	

WO 9745000	A	04-12-1997	AU 1142397 A	05-01-1998
			CA 2187728 A	29-11-1997
			US 5846821 A	08-12-1998

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			US 5965792 A	12-10-1999
